

The use of corneas from animals of different age in the Bovine Corneal Opacity and Permeability (BCOP) assay.

Freddy Van Goethem, Marc Sysmans and Philippe Vanparys

Johnson & Johnson Pharmaceutical Research & Development, a division of Janssen Pharmaceutica N.V.,
Genetic and In Vitro Toxicology, Turnhoutseweg 30, B-2340 Beerse, Belgium.

BCOP results obtained with corneas from:

- 1) adult animals (> 24 months)
- 2) young animals (6 - 8 months)

Methodology

After background opacity measurement, medium was removed from the anterior compartment and corneas were treated with 0.75 ml of the test solution. Corneas (3 per group) were treated for 10 minutes followed by a 120 minutes recovery period. Medium was removed from the anterior compartment and replaced by 1 ml of a 0.4% sodium-fluorescein solution. Corneas were incubated in a horizontal position for 90 minutes at 32°C in a water-bath. After incubation, medium from the posterior chamber was removed and its optical density (OD) determined with a spectrophotometer at 490 nm. In Vitro Score = opacity + [15 x permeability]

Code of each compound is recorded on each raw data sheet

>>> compound 17 (acetone) need to be repeated since results did not comply with previously collected data in our laboratory. Due to the high vapor pressure of acetone (201.57 mmHg @ 22.0 °C), a technical artefact could have occurred...

The use of corneas from animals of different age in the Bovine Corneal Opacity and Permeability (BCOP) assay.

Code	Compound	CAS No.	In vivo EU	In vivo GHS	In Vitro BCOP (>24 months)				In Vitro BCOP (6 - 8 months)			
					Opacity	Perm.	IVS	Class	Opacity	Perm.	IVS	Class
1	3,3-dimethylpentane	562-49-2	NI	NI	0.6	0.01	0.8	NON	0.0	0.02	0.3	NON
2	3-methoxy-1,2-propanediol	623-39-2	NI	NI	-0.3	0.00	0.2	NON	0.6	0.02	0.9	NON
3	polyethylene glycol 400	25322-68-3	NI	NI	-0.3	0.00	-0.3	NON	0.0	0.08	1.1	NON
4	glycerol	56-81-5	NI	NI	-1.0	0.01	-0.9	NON	-0.7	-0.01	-0.8	NON
5	methyl cyclopentane	96-37-7	NI	NI	1.0	0.43	7.5	MILD	1.3	0.26	5.2	MILD
6	tween 20	9005-64-5	NI	NI	0.0	0.01	0.1	NON	0.0	-0.01	-0.1	NON
7	methyl <i>iso</i> -butyl ketone	108-10-1	NI	NI	6.6	1.07	22.7	MILD	5.7	0.83	18.1	MILD
8	toluene	108-88-3	NI	NI	6.3	3.18	54	MOD	6.0	1.46	28.0	MOD
9	methyl amyl ketone	110-43-0	NI	NI	5.3	1.80	32.3	MOD	4.0	0.99	18.8	MILD
10	2-methyl-1-pentanol	105-30-6	NI	2B	12.0	4.30	76.6	SEV	8.6	1.94	37.7	MOD
11	ethanol	64-17-5	NI	2B	16.0	2.34	51	MOD	16.3	1.83	43.8	MOD
12	sodium hydroxide (1%)	1310-73-2	R36	2B	99.7	4.16	162	SEV	135.7	3.74	191.8	SEV
13	triton X-100 (5%)	9002-93-1	R36	2B	4.3	3.81	61.5	SEV	4.7	3.70	60.1	SEV
14	1-octanol	111-87-5	R36	2B	10.0	5.24	88.6	SEV	10.3	1.53	33.3	MOD
15	2-ethyl-1-hexanol	104-76-7	R36	2B	4.3	1.76	30.6	MOD	2.3	0.86	15.3	MILD
16	n-hexanol	111-27-3	R36	2A	15.3	3.73	71.2	SEV	14.0	3.62	68.2	SEV
17	acetone	67-64-1	R36	2A	39**	2.95	83.2	SEV	91.3	2.86	134.2	SEV
18	cyclohexanol	108-93-0	R41	1	15.3	5.04	90.7	SEV	11.6	2.13	43.6	MOD
19	cetylpyridinium bromide (6%)	140-72-7	R41	1	11.7	1.01	26.8	MOD	15.0	1.66	39.9	MOD
20	benzalkonium chloride (10%)	8001-54-5	R41	1	92.2	4.22	155.4	SEV	105.7	4.05	166.5	SEV

Prediction Model

BCOP In Vitro Score	Class
≤ 3	NON
3.1-25	MILD
25.1-55	MOD
> 55.1	SEV

** to be repeated (technical artefact probably occurred)

- Compounds 1 → 20
- Adult animals (> 24 months)

Calculation of the in vitro eye irritation score for liquids

Test article	3,3-Dimethylpentane [562-49-2]		
Batch No.	14502CN		
Concentration	100%	Treatment time	10 min
Code	A1	OP-KIT	
Sequence	Intern 8B		

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	2	2	0	0.006	0.1
2	MEM	1	1	0	0.012	0.2
3	100%	1	3	2	0.009	2.1
Mean ± S.D.		0.7 ± 1.2			0.009 ± 0.003	0.8 ± 1.1
					Corrected value	
4	Test article	1	3	2	0.023	1.5
5	100%	0	1	1	0.018	0.4
6		1	2	1	0.014	0.4
Mean ± S.D.		0.6 ± 0.6			0.009 ± 0.005	0.8 ± 0.6

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	75 B	-75
	2	154 B	-157
	3	250 B	-255

Paraph

Date 18-Jan-00

Calculation of the in vitro eye irritation score for liquids

Test article	3-methoxy-1,2-propanediol 98%		
Batch No.	05307-078		
Concentration	100%	Treatment time	2 hours
Code	B1 (2)		
Sequence	Intern 10A	OP-KIT	

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.004	0.1
2	MEM	0	0	0	0.008	0.1
3	100%	0	1	1	0.010	1.2
Mean ± S.D.		0.3 ± 0.6			0.007 ± 0.003	0.5 ± 0.6
		Corrected value			Corrected value	
4	Test article	0	0	0	0.019	0.012
5		0	0	0	0.066	0.059
6	100%	0	0	0	0.032	0.025
Mean ± S.D.		-0.3 ± 0.0			0.032 ± 0.024	0.2 ± 0.4

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A	B
	2	A	B
	3	A	B

Paraph

Date 31-Jan-00

Calculation of the in vitro eye irritation score for liquids

Test article	Polyethylene glycol 400		
Batch No.	3H0110		
Concentration	100%	Treatment time	10 min
Code	C1 (3)		
Sequence	11A	OP-KIT	

No. Cornea	Treatment	Opacity at			Permeability	In vitro score	
		t0	t120	t120 - t0			
1	NC	0	0	0	0.002	0.0	
2	NaCl 0.9%	0	0	0	0.003	0.0	
3	100%	1	1	0	0.001	0.0	
Mean ± S.D.		0.0 ± 0.0			0.002 ± 0.001	0.0 ± 0.0	
					Corrected value	Corrected value	
4	Test article	0	0	0	0.000	-0.002	0.0
5	100%	0	0	0	0.003	0.001	0.0
6		1	0	-1	0.010	0.008	-0.9
Mean ± S.D.		-0.3 ± 0.6			0.002 ± 0.005	-0.3 ± 0.5	

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A	B
	2	A	B
	3	A	B

Paraph

Date 28-Feb-00

Calculation of the in vitro eye irritation score for liquids

Test article	Glycerol		
Batch No.	HS03116BS		
Concentration	100%	Treatment time	10 min
Code	B2 (4)		
Sequence	Intern 10A	OP-KIT	

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.004	0.1
2	MEM	0	0	0	0.008	0.1
3	100%	0	1	1	0.010	1.2
Mean ± S.D.		0.3 ± 0.6			0.007 ± 0.003	0.5 ± 0.6
					Corrected value	Corrected value
7	Test article	1	0	-1	-1.3	-1.2
8	100%	1	1	0	-0.3	-0.2
9		1	0	-1	-1.3	-1.2
Mean ± S.D.		-1.0 ± 0.6			0.009 ± 0.001	-0.9 ± 0.6

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A	B
	2	A	B
	3	A	B

Paraph

Date

Calculation of the in vitro eye irritation score for liquids

Test article	Methyl cyclopentane		
Batch No.	09817PS-089		
Concentration	100%	Treatment time	10 min
Code	D5	OP-KIT	
Sequence	12A		

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.004	0.1
2	NaCl 0.9%	1	1	0	0.006	0.1
3	100%	1	1	0	0.005	0.1
Mean ± S.D.		0.0 ± 0.0			0.005 ± 0.001	0.1 ± 0.0
					Corrected value	
16	Test article	0	1	1	1.0	4.9
17	100%	0	2	2	2.0	14.5
18		0	0	0	0.0	3.1
Mean ± S.D.		1.0 ± 1.0			0.433 ± 0.348	7.5 ± 6.1
					Corrected value	

NC: Negative Control

REMARKS	Filter		OPACITY	
	1	A		B
	2	A		B
	3	A		B

Paraph 20-Mar-00

Date

Calculation of the in vitro eye irritation score for liquids

Test article	Tween 20		
Batch No.	A010055102		
Concentration	100%	Treatment time	10 min
Code	C2 6		
Sequence	11A	OP-KIT	

No.	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.002	0.0
2	NaCl 0.9%	0	0	0	0.003	0.0
3	100%	1	1	0	0.001	0.0
Mean ± S.D.		0.0 ± 0.0			0.002 ± 0.001	0.0 ± 0.0
					Corrected value	
7	Test article	0	0	0	0.010	0.1
8	100%	0	0	0	0.023	0.3
9		0	0	0	0.004	0.0
Mean ± S.D.		0.0 ± 0.0			0.010 ± 0.010	0.1 ± 0.2

NC: Negative Control

REMARKS	Filter		OPACITY	
	1	A		B
	2	A		B
	3	A		B

Paraph

Date

Calculation of the in vitro eye irritation score for liquids

Test article	Methyl iso-butyl ketone (4 methyl-2-pentanone) [108-10-1]		
Batch No.	CU 10369BU		
Concentration	100%	Treatment time	10 min
Code	A2 (7)		
Sequence	Intern 8B	OP-KIT	

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	2	2	0	0.006	0.1
2	MEM	1	1	0	0.012	0.2
3	100%	1	3	2	0.009	2.1
Mean ± S.D.		0.7 ± 1.2			0.009 ± 0.003	0.8 ± 1.1
					Corrected value	
7	Test article	1	8	7	6.3	34.8
8	100%	0	7	7	6.3	15.5
9		1	9	8	7.3	17.8
Mean ± S.D.		6.6 ± 0.6			1.070 ± 0.720	22.7 ± 10.5

NC: Negative Control

REMARKS	Filter		OPACITY	
	1	A		B
	2	A		B
	3	A		B

Paraph

Date 18-Jan-00

Calculation of the in vitro eye irritation score for liquids

Test article	Toluene [108-88-3]		
Batch No.	990281O001		
Concentration	100%	Treatment time	
Code	D4 (8)		
Sequence	12A		OP-KIT

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.004	0.1
2	NaCl 0.9%	1	1	0	0.006	0.1
3	100%	1	1	0	0.005	0.1
	Mean ± S.D.	0.0 ± 0.0			0.005 ± 0.001	0.1 ± 0.0
		Corrected value			Corrected value	
13	Test article	0	6	6	6.0	58.1
14		0	7	7	7.0	49.4
15	100%	0	6	6	6.0	54.5
	Mean ± S.D.	6.3 ± 0.6			3.178 ± 0.327	54.0 ± 4.4

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A	B
	2	A	B
	3	A	B

Paraph

Date 20-Mar-00

Calculation of the in vitro eye irritation score for liquids

Test article	methyl amyl ketone (2 heptanone) [110-43-0]		
Batch No.	66400-104		
Concentration	100%	Treatment time	10 min
Code	A3	OP-KIT	
Sequence	Intern 8B		

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	2	2	0	0.006	0.1
2	MEM	1	1	0	0.012	0.2
3	100%	1	3	2	0.009	2.1
Mean ± S.D.		0.7 ± 1.2			0.009 ± 0.003	0.8 ± 1.1
					Corrected value	Corrected value
10	Test article	1	8	7	6.3	22.6
11	100%	0	6	6	5.3	34.0
12		2	7	5	4.3	40.2
Mean ± S.D.		5.3 ± 1.0			1.799 ± 0.662	32.3 ± 8.9

NC: Negative Control

REMARKS	Filter		OPACITY	
	1	A		B
	2	A		B
	3	A		B

Paraph

Date 18-Jan-00

Calculation of the in vitro eye irritation score for liquids

Test article	2-methyl-1-pentanol		
Batch No.	05002PG		
Concentration	100%	Treatment time	10 min
Code	B3	OP-KIT	
Sequence	Intern 10A		

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.004	0.1
2	MEM	0	0	0	0.008	0.1
3	100%	0	1	1	0.010	1.2
Mean ± S.D.		0.3 ± 0.6			0.007 ± 0.003	0.5 ± 0.6
					Corrected value	
10	Test article	0	10	10	9.7	59.6
11	100%	0	13	13	12.7	86.3
12		0	14	14	13.7	83.8
Mean ± S.D.		12.0 ± 2.1			4.304 ± 0.852	76.6 ± 14.7
					Corrected value	
10	Test article	0	10	10	3.336	3.329
11	100%	0	13	13	4.916	4.909
12		0	14	14	4.680	4.673
Mean ± S.D.		12.0 ± 2.1			4.304 ± 0.852	76.6 ± 14.7

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A	B
	2	A	B
	3	A	B

Paraph

Date 31-Jan-00

Calculation of the in vitro eye irritation score for liquids

Test article	Ethanol [64-17-5]		
Batch No.	993O710002		
Concentration	100%	Treatment time	10 min
Code	D1	OP-KIT	
Sequence	12A		

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.004	0.1
2	NaCl 0.9%	1	1	0	0.006	0.1
3	100%	1	1	0	0.005	0.1
Mean ± S.D.		0.0 ± 0.0			0.005 ± 0.001	0.1 ± 0.0
					Corrected value	Corrected value
4	Test article	0	16	16	2.340	51.0
5	100%	0	17	17	2.164	49.4
6		0	15	15	2.520	52.7
Mean ± S.D.		16.0 ± 1.0			2.336 ± 0.178	51.0 ± 1.7

NC: Negative Control

REMARKS		Filter	OPACITY	
	1			
	2			
	3			

Paraph

Date 20-Mar-00

Calculation of the in vitro eye irritation score for liquids

Test article	Sodium hydroxide 1%		
Batch No.	66H0320		
Concentration	1%	Treatment time	
Code	D3		
Sequence	12A		OP-KIT

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.004	0.1
2	NaCl 0.9%	1	1	0	0.006	0.1
3	100%	1	1	0	0.005	0.1
Mean ± S.D.		0.0 ± 0.0			0.005 ± 0.001	0.1 ± 0.0
					Corrected value	Corrected value
10	Test article	0	101	101	101.0	160.2
11		0	111	111	111.0	175.1
12	100%	0	87	87	87.0	150.8
Mean ± S.D.		99.7 ± 12.1			4.156 ± 0.182	162.0 ± 12.3

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A	B
	2	A	B
	3	A	B

Paraph

Date 20-Mar-00

Calculation of the in vitro eye irritation score for liquids

Test article	Triton X-100 (5%)	
Batch No.	28H2536	
Concentration	100%	Treatment time 10 min
Code	C4 (13)	
Sequence	11A	OP-KIT

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.002	0.0
2	NaCl 0.9%	0	0	0	0.003	0.0
3	100%	1	1	0	0.001	0.0
Mean ± S.D.		0.0 ± 0.0			0.002 ± 0.001	0.0 ± 0.0
		Corrected value			Corrected value	
13	Test article	1	6	5	5.0	69.0
14		0	4	4	4.0	54.7
15	100%	2	6	4	4.0	60.9
Mean ± S.D.		4.3 ± 0.6			3.813 ± 0.442	61.5 ± 7.2

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A	B
	2	A	B
	3	A	B

Paraph _____

Date 28-Feb-00

Calculation of the in vitro eye irritation score for liquids

Test article	n-octanol		
Batch No.	27336-019		
Concentration	100%	Treatment time	10 min
Code	B4	14	
Sequence	Intern 10A	OP-KIT	

No. Cornea	Treatment	Opacity at			Permeability	In vitro score	
		t0	t120	t120 - t0			
1	NC	0	0	0	0.004	0.1	
2	MEM	0	0	0	0.008	0.1	
3	100%	0	1	1	0.010	1.2	
Mean ± S.D.		0.3 ± 0.6			0.007 ± 0.003	0.5 ± 0.6	
Corrected value					Corrected value		
13	Test article	1	7	6	5.180	5.173	83.3
14	100%	0	15	15	5.828	5.821	102.0
15		1	11	10	4.724	4.717	80.5
Mean ± S.D.		10.0 ± 4.5			5.237 ± 0.555		88.6 ± 11.7

NC: Negative Control

REMARKS	Filter		OPACITY	
	1	A		B
	2	A		B
	3	A		B

Paraph

Date 31-Jan-00

Calculation of the in vitro eye irritation score for liquids

Test article	2-ethyl-1-hexanol [107-76-7]		
Batch No.	26812-019		
Concentration	100%	Treatment time	10 min
Code	A4 (15)		
Sequence	Intern 8B	OP-KIT	

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	2	2	0	0.006	0.1
2	MEM	1	1	0	0.012	0.2
3	100%	1	3	2	0.009	2.1
Mean ± S.D.		0.7 ± 1.2			0.009 ± 0.003	0.8 ± 1.1
					Corrected value	Corrected value
13	Test article	2	6	4	3.3	29.6
14	100%	1	7	6	5.3	38.1
15		2	7	5	4.3	24.2
Mean ± S.D.		4.3 ± 1.0			1.756 ± 0.430	30.6 ± 7.0

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A	B
	2	A	B
	3	A	B

Paraph

Date 18-Jan-00

Calculation of the in vitro eye irritation score for liquids

Test article	1-Hexanol [111-27-3]		
Batch No.	381949/1		
Concentration	100%	Treatment time	
Code	D2		
Sequence	12A		OP-KIT

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.004	0.1
2	NaCl 0.9%	1	1	0	0.006	0.1
3	100%	1	1	0	0.005	0.1
Mean ± S.D.		0.0 ± 0.0			0.005 ± 0.001	0.1 ± 0.0
					Corrected value	
7	Test article	0	17	17	17.0	72.4
8	100%	0	13	13	13.0	73.8
9		0	16	16	16.0	67.5
Mean ± S.D.		15.3 ± 2.1			3.728 ± 0.311	71.2 ± 3.3
					Corrected value	
		3.700	3.695			
		4.060	4.055			
		3.440	3.435			

NC: Negative Control

REMARKS	Filter	OPACITY		
	1	A	B	
	2	A	B	
	3	A	B	

Paraph

Date 20-Mar-00

Calculation of the in vitro eye irritation score for liquids

Test article	Acetone [67-64-1]		
Batch No.	39H3430		
Concentration	100%	Treatment time	10 min
Code	A5 17	OP-KIT	
Sequence	Intern 8B		

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	2	2	0	0.006	0.1
2	MEM	1	1	0	0.012	0.2
3	100%	1	3	2	0.009	2.1
Mean ± S.D.		0.7 ± 1.2			0.009 ± 0.003	0.8 ± 1.1
Corrected value					Corrected value	
16	Test article	1	36	35	34.3	59.5
17		1	42	41	40.3	83.5
18	100%	1	44	43	42.3	106.7
Mean ± S.D.		39.0 ± 4.2			2.951 ± 1.309	83.2 ± 23.6

NC: Negative Control

REMARKS	Filter		OPACITY	
	1	A	B	
	2	A	B	
	3	A	B	

Paraph

Date 18-Jan-00

Calculation of the in vitro eye irritation score for liquids

Test article	Cyclohexanol		
Batch No.	18285-049		
Concentration	100%	Treatment time	10 min
Code	B5	(18)	
Sequence	Intern 10A	OP-KIT	

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.004	0.1
2	MEM	0	0	0	0.008	0.1
3	100%	0	1	1	0.010	1.2
Mean ± S.D.		0.3 ± 0.6			0.007 ± 0.003	0.5 ± 0.6
					Corrected value	Corrected value
16	Test article	0	16	16	6.180	108.3
17		0	16	16	3.288	64.9
18	100%	0	14	14	5.680	98.8
Mean ± S.D.		15.0 ± 1.2			5.042 ± 1.546	90.7 ± 22.8

NC: Negative Control

REMARKS		Filter	OPACITY	
	1	A _t		
	2	A _t		
	3	A _t		

Paraph

Eate 31-Jan-00

Calculation of the in vitro eye irritation score for liquids

Test article	Cetylpyridinium bromide (6%)		
Batch No.	105H0915		
Concentration	100%	Treatment time	
Code	C5	(19)	
Sequence	11A	OP-KIT	

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.002	0.0
2	NaCl 0.9%	0	0	0	0.003	0.0
3	100%	1	1	0	0.001	0.0
Mean ± S.D.		0.0 ± 0.0			0.002 ± 0.001	0.0 ± 0.0
		Corrected value			Corrected value	
16	Test article	1	15	14	14.0	38.9
17	100%	1	12	11	11.0	20.8
18		0	10	10	10.0	20.7
Mean ± S.D.		11.7 ± 2.1			1.008 ± 0.563	26.8 ± 10.5

NC: Negative Control

REMARKS		OPACITY	
	Filter		
	1	A	B
	2	A	B
	3	A	B

Paraph

Date 28-Jan-00

Benzalkonium chloride (10%)

Exp.	Opacity	Permeability	In Vitro Score
1	88.0	4.426	154.4
2	94.6	4.148	156.9
3	87.0	4.252	150.8
4	93.0	4.278	157.2
5	98.3	3.972	157.9
mean	92.2	4.2	155.4
SD	4.7	0.17	2.9

BCOP PREVALIDATION 1997

Calculation of in vitro eye irritation score for surfactants (10% w/w)

Test article	1 (BAK)		
Batch No.	76H2520		
Concentration	10 g/g%	Treatment time	10 min
Prevalidation phase	II		
Sequence	A		

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
16	NC	1	2	1	0.005	1.1
17	0.9 % NaCl	1	2	1	0.018	1.3
18	100%	1	2	1	0.002	1.0
Mean ± S.D.		1.0 ± 0.0			0.008 ± 0.009	1.1 ± 0.2
					Corrected value	
19	Test article	1	97	96	95.0	153.8
20		1	82	81	80.0	143.6
21		10g/g%	1	91	90	89.0
Mean ± S.D.		88.0 ± 7.5			4.426 ± 0.623	154.4 ± 11.1

NC: Negative control
PC: Positive control

REMARKS	Filter		OPACITY	
	1	A	75	B -75
	2	A	153	B -159
	3	A	236	B -253

Paraph

Date 13-Feb-97

BCOP PREVALIDATION 1997

Calculation of in vitro eye irritation score for surfactants (10% w/w)

Test article	I (BAK)		
Batch No.	76H2520		
Concentration	10 g/g%	Treatment time	10 min
Prevalidation phase	II		
Sequence	D		

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
16	NC	1	1	0	0.005	0.1
17	0.9 % NaCl	1	2	1	0.007	1.1
18	100%	0	1	1	0.004	1.1
Mean ± S.D.		0.7 ± 0.6			0.005 ± 0.002	0.8 ± 0.6
					Corrected value	
19	Test article	1	108	107	106.3	178.0
20	10g/g%	0	92	92	91.3	143.2
21		0	87	87	86.3	149.4
Mean ± S.D.		♦ 94.6 ± 10.4			4.148 ± 0.662	156.9 ± 18.6

NC: Negative control
 PC: Positive control

REMARKS	Filter		OPACITY	
	1	A	75	B -75
	2	A	153	B -158
	3	A	235	B -252

Paraph

Date

BCOP PREVALIDATION 1997

Calculation of in vitro eye irritation score for surfactants (10% w/w)

Test article	1 (BAK)		
Batch No.	76H2520		
Concentration	10 g/g%	Treatment time	10 min
Prevalidation phase	II		
Sequence	F		

No.	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
10	NC	0	1	1	0.009	1.1
11	0.9 % NaCl	1	2	1	0.001	1.0
12	100%	0	1	1	0.018	1.3
	Mean ± S.D.			1.0 ± 0.0	0.009 ± 0.009	1.1 ± 0.2

No.	Test article	Opacity at			Corrected value		Corrected value	In vitro score	
		t0	t120	t120 - t0					
19	10g/g%	1	88	87	86.0		4.333	4.324	150.9
20		1	82	81	80.0		4.255	4.246	143.7
21		1	97	96	95.0		4.196	4.187	157.8
	Mean ± S.D.				87.0 ± 7.5		4.252 ± 0.069		150.8 ± 7.1

NC: Negative control
PC: Positive control

REMARKS	Filter		OPACITY	
	1	A	75	B -75
	2	A	152	B -158
	3	A	234	B -252

Paraph

Date 13-Mar-97

BCOP PREVALIDATION 1997

Calculation of in vitro eye irritation score for surfactants (10% w/w)

Test article	I (BAK)		
Batch No.	76H2520		
Concentration	10 g/g%	Treatment time	10 min
Prevalidation phase	II		
Sequence	G		

No.	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
19	NC	3	3	0	0.008	0.1
20	0.9 % NaCl	0	0	0	0.038	0.6
21	100%	1	1	0	0.012	0.2
Mean ± S.D.		0.0 ± 0.0			0.019 ± 0.016	0.3 ± 0.3
					Corrected value	Corrected value
25	Test article	0	96	96	96.0	163.7
26		0	93	93	93.0	171.0
27	10g/g%	2	92	90	90.0	136.8
Mean ± S.D.		93.0 ± 3.0			4.278 ± 1.058	157.2 ± 18.0

NC: Negative control
 PC: Positive control

REMARKS	Filter		OPACITY	
	1	A	75	B -75
	2	A	152	B -158
	3	A	231	B -249

Paraph

Date 20-Mar-97

BCOP PREVALIDATION 1997

Calculation of in vitro eye irritation score for surfactants (10% w/w)

Test article	1 (BAK)		
Batch No.	76H2520		
Concentration	10 g/g%	Treatment time	10 min
Prevalidation phase	II		
Sequence	F H		

No.	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
10	NC	1	1	0	0.013	0.2
11	0.9 % NaCl	1	4	3	0.013	3.2
12	100%	1	1	0	0.021	0.3
Mean ± S.D.		1.0 ± 1.7			0.016 ± 0.005	1.2 ± 1.7

No.	Test article	Opacity at			Corrected value		Permeability	Corrected value		In vitro score
		t0	t120	t120 - t0						
19	10g/g%	1	99	98	97.0		4.051	4.035	157.5	
20		1	99	98	97.0		4.312	4.296	161.4	
21		1	103	102	101.0		3.600	3.584	154.8	
Mean ± S.D.					98.3 ± 2.3		3.972 ± 0.360		157.9 ± 3.3	

NC: Negative control
 PC: Positive control

REMARKS	Filter	OPACITY		
	1	A	75	B -75
	2	A	152	B -161
	3	A	236	B -253

Paraph

Date 21-Mar-97

• Compounds 1 → 20

• young animals (6-8 months)

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	3,3 Dimethylpentane [562-49-2]		
Batch No.	14602CN		
Concentration	99%	Treatment time	10 min
Code	A1		
Sequence	2005/ Intern3 kalveren	OP-KIT	

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.008	0.1
2	MEM	0	0	0	0.026	0.4
3	100%	0	0	0	0.006	0.1
Mean ± S.D.		0.0 ± 0.0			0.013 ± 0.011	0.2 ± 0.2
					Corrected value	
4	Test article	0	0	0	0.046	0.5
5		0	0	0	0.028	0.2
6	100%	0	0	0	0.023	0.2
Mean ± S.D.		0.0 ± 0.0			0.019 ± 0.012	0.3 ± 0.2

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A 75	B -75
	2	A 158	B -160
	3	A 256	B -258

Paraph		Filter	
		0.1	1
		0.3	15
Date	07-Mar-05	0.6	50
		0.8	90
		1	145

RDF/BCO/18

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	3-methoxy-1,2-propanediol [623-39-2]		
Batch No.	A0155893001		
Concentration	100%	Treatment time	10 min
Code	B1		
Sequence	2005/ intern2 kalveren		OP-KIT

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.084	1.3
2	NaCl 0.9%	0	2	2	0.085	3.3
3	100%	1	1	0	0.036	0.5
Mean ± S.D.		0.7 ± 1.2			0.068 ± 0.028	1.7 ± 1.4
					Corrected value	Corrected value
4	Test article	0	0	0	0.090	-0.4
5		2	6	4	0.096	3.7
6	100%	0	0	0	0.070	-0.7
Mean ± S.D.		0.6 ± 2.3			0.017 ± 0.014	0.9 ± 2.5

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A 75	B -75
	2	A 155	B -161
	3	A 259	B -261

Paraph		Filter		
		0.1	1	
		0.3	16	
Date	01-Mar-05	0.6	51	
		0.8	91	
		1	143	

RDF/BCO/18

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	polyethylene glycol 400 [25322-68-3]		
Batch No.	S23152-394		
Concentration	100%	Treatment time	10 min
Code	C1 (3)		
Sequence	2005/ intern 1 kalveren	OP-KIT	

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.023	0.3
2	NaCl 0.9%	0	0	0	0.069	1.0
3	100%	0	0	0	0.044	0.7
Mean ± S.D.		0.0 ± 0.0			0.045 ± 0.023	0.7 ± 0.4
					Corrected value	
4	Test article	0	0	0	0.102	0.9
5	100%	0	0	0	0.178	2.0
6		0	0	0	0.080	0.5
Mean ± S.D.		0.0 ± 0.0			0.075 ± 0.051	1.1 ± 0.8

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A	75 B -75
	2	A	157 B -161
	3	A	260 B -259

Paraph	Filter	
	0.1	1
	0.3	16
Date	0.6	50
	0.8	88
	1	140

RDF/BCO/18

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	glycerol [56-81-5]		
Batch No.	13574HC		
Concentration	100%	Treatment time	10 min
Code	B2 (4)		
Sequence	2005/ intern2 kalveren	OP-KIT	

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.084	1.3
2	NaCl 0.9%	0	2	2	0.085	3.3
3	100%	1	1	0	0.036	0.5
Mean ± S.D.		0.7 ± 1.2			0.068 ± 0.028	1.7 ± 1.4
					Corrected value	Corrected value
7	Test article	0	0	0	0.008	-1.6
8	100%	0	0	0	0.009	-1.6
9		2	2	0	0.161	0.7
Mean ± S.D.		-0.7 ± 0.0			-0.009 ± 0.088	-0.8 ± 1.3

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A	75 B -75
	2	A	155 B -161
	3	A	259 B -261

Paraph		Filter		
		0.1	1	
		0.3	16	
Date	01-Mar-05	0.6	51	
		0.8	91	
		1	143	

RDF/BCO/18

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	Methyl cyclopentane [96-37-7]		
Batch No.	1097605		
Concentration	95%	Treatment time	10 min
Code	D5	5	
Sequence	2005/ Intern3 kalveren	OP-KIT	

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.008	0.1
2	NaCl 0.9%	0	0	0	0.026	0.4
3	100%	0	0	0	0.006	0.1
Mean ± S.D.		0.0 ± 0.0			0.013 ± 0.011	0.2 ± 0.2
					Corrected value	Corrected value
16	Test article	0	2	2	2.0	8.5
17		1	2	1	1.0	3.5
18	100%	0	1	1	1.0	3.7
Mean ± S.D.		1.3 ± 0.6			0.260 ± 0.149	5.2 ± 2.8

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A	75 B -75
	2	A	158 B -160
	3	A	256 B -258

Paraph		Filter			
		0.1	1		
		0.3	15		
Date	07-Mar-05	0.6	50		
		0.8	90		
		1	145		

RDF/BCO/18

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	Tween 20 {9005-64-5}		
Batch No.	094K01761		
Concentration	100%	Treatment time	10 min
Code	C2		
Sequence	2005/Intern4 kalverogen		OP-KIT

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.045	0.7
2	NaCl 0.9%	0	0	0	0.022	0.3
3	100%	0	0	0	0.012	0.2
Mean ± S.D.		0.0 ± 0.0			0.026 ± 0.017	0.4 ± 0.3
					Corrected value	Corrected value
19	Test article	0	0	0	0.028	0.0
20		0	0	0	0.021	-0.1
21	100%	0	0	0	0.013	-0.2
Mean ± S.D.		0.0 ± 0.0			-0.005 ± 0.008	-0.1 ± 0.1

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A 75	B -75
	2	A 156	B -158
	3	A 263	B -258

Paraph		Filter			
			0.1	0	
			0.3	15	
Date	14-Mar-05		0.6	50	
			0.8	89	
			1	141	

RDF/BCO/18

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	Methyl iso-butyl ketone (4 methyl-2-pentanone) [108-10-1]		
Batch No.	1127250		
Concentration	100%	Treatment time	10 min
Code	A2 (7)		
Sequence	2005/ internI kalveren	OP-KIT	

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.023	0.3
2	NaCl 0.9%	0	0	0	0.069	1.0
3	100%	0	0	0	0.044	0.7
Mean ± S.D.		0.0 ± 0.0			0.045 ± 0.023	0.7 ± 0.4
					Corrected value	
7	Test article	0	3	3	0.714	13.0
8	100%	0	8	8	0.861	20.2
9		0	6	6	1.059	21.2
Mean ± S.D.		5.7 ± 2.5			0.833 ± 0.173	18.1 ± 4.5

NC: Negative Control

REMARKS	Filter	OPACITY
	1	A 75 B -75
	2	A 157 B -161
	3	A 260 B -259

Paraph	Filter	Value
	0.1	1
	0.3	16
Date	0.6	50
	0.8	88
	1	140

RDF/BCO/18

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	Toluene [108-88-3]		
Batch No.	A0204558001		
Concentration	100%	Treatment time	10 min
Code	D4	8	
Sequence	2005/ Intern3 kalveren	OP-KIT	

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.008	0.1
2	NaCl 0.9%	0	0	0	0.026	0.4
3	100%	0	0	0	0.006	0.1
Mean ± S.D.		0.0 ± 0.0			0.013 ± 0.011	0.2 ± 0.2
					Corrected value	Corrected value
13	Test article	0	2	2	2.0	25.1
14	100%	0	9	9	9.0	36.6
15		0	7	7	7.0	22.3
Mean ± S.D.		6.0 ± 3.6			1.464 ± 0.416	28.0 ± 7.6

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A 75 B	-75
	2	A 158 B	-160
	3	A 256 B	-258

Paraph		Filter		
		0.1	1	
		0.3	15	
Date	07-Mar-05	0.6	50	
		0.8	90	
		1	145	

RDF/BCO/18

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	methyl amyl ketone (2 heptanone) [110-43-0]		
Batch No.	13622JC		
Concentration	100%	Treatment time	10 min
Code	A3 (9)		
Sequence	2005/ intern 1 kalveren	OP-KIT	

No. Cornea	Treatment	Opacity at			Permeability	In vitro score			
		t0	t120	t120 - t0					
1	NC	0	0	0	0.023	0.3			
2	NaCl 0.9%	0	0	0	0.069	1.0			
3	100%	0	0	0	0.044	0.7			
Mean ± S.D.		0.0 ± 0.0			0.045 ± 0.023	0.7 ± 0.4			
		Corrected value				Corrected value			
10	Test article	0	5	5	5.0	1.065	1.020	20.3	
11	100%	0	5	5	5.0	1.030	0.985	19.8	
12		2	4	2	2.0	0.995	0.950	16.3	
Mean ± S.D.		4.0 ± 1.7				0.985 ± 0.035		18.8 ± 2.2	

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A 75	B -75
	2	A 157	B -161
	3	A 260	B -259

Paraph		Filter		
		0.1	1	
		0.3	16	
Date	28-Feb-05	0.6	50	
		0.8	88	
		1	140	

RDF/BCO/18

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	2-methyl-1-pentanol [105-30-6]		
Batch No.	451942/1		
Concentration	100%	Treatment time	10 min
Code	B3	10	
Sequence	2005/ intern 2 kalveren	OP-KIT	

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.084	1.3
2	NaCl 0.9%	0	2	2	0.085	3.3
3	100%	1	1	0	0.036	0.5
Mean ± S.D.		0.7 ± 1.2			0.068 ± 0.028	1.7 ± 1.4
					Corrected value	Corrected value
10	Test article	0	11	11	10.3	36.3
11		0	8	8	7.3	42.8
12	100%	0	9	9	8.3	33.9
Mean ± S.D.		8.6 ± 1.5			1.935 ± 0.375	37.7 ± 4.6

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A	75 B -75
	2	A	155 B -161
	3	A	259 B -261

Paraph		Filter			
		0.1		1	
		0.3		16	
Date	01-Mar-05	0.6		51	
		0.8		91	
		1		143	

RDF/BCO/18

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	Ethanol [64-17-5]		
Batch No.	K33957583 448		
Concentration	100%	Treatment time	10 min
Code	D1	<i>(Handwritten mark)</i>	
Sequence	2005/ Intern3 kalveren	OP-KIT	

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.008	0.1
2	NaCl0.9%	0	0	0	0.026	0.4
3	100%	0	0	0	0.006	0.1
Mean ± S.D.		0.0 ± 0.0			0.013 ± 0.011	0.2 ± 0.2
					Corrected value	Corrected value
7	Test article	0	18	18	18.0	52.4
8		0	16	16	16.0	41.3
9		100%	0	15	15	15.0
Mean ± S.D.		16.3 ± 1.5			1.834 ± 0.408	43.8 ± 7.6

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A 75	B -75
	2	A 158	B -160
	3	A 256	B -258

Paraph	Filter
	0.1 1
	0.3 15
Date 07-Mar-05	0.6 50
	0.8 90
	1 145

RDF/BCO/18

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	Sodium hydroxide 1% [1310-73-2]		
Batch No.	014K0006		
Concentration	1%	Treatment time	10 min
Code	D3	(12)	
Sequence	2005/Intern4 kalverogen	OP-KIT	

No.	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.045	0.7
2	NaCl 0.9%	0	0	0	0.022	0.3
3	100%	0	0	0	0.012	0.2
Mean ± S.D.		0.0 ± 0.0			0.026 ± 0.017	0.4 ± 0.3
Corrected value					Corrected value	
16	Test article	0	139	139	139.0	206.7
17		0	145	145	145.0	183.6
18	100%	0	123	123	123.0	185.1
Mean ± S.D.		135.7 ± 11.4			3.742 ± 1.029	191.8 ± 12.9

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A 75	B -75
	2	A 156	B -158
	3	A 263	B -258

Filter	
0.1	0
0.3	15
0.6	50
0.8	89
1	141

Paraph

Date 14-Mar-05

RDF/BCO/18

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	Triton X-100 (5%) [9002-93-1]		
Batch No.	A019437801		
Concentration	5%	Treatment time	10 min
Code	C4 (13)		
Sequence	2005/Intern4 kalverogen	OP-KIT	

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.045	0.7
2	NaCl 0.9%	0	0	0	0.022	0.3
3	100%	0	0	0	0.012	0.2
Mean ± S.D.		0.0 ± 0.0			0.026 ± 0.017	0.4 ± 0.3
Corrected value					Corrected value	
10	Test article	0	5	5	5.0	69.1
11		0	5	5	5.0	49.7
12	100%	0	4	4	4.0	61.5
Mean ± S.D.		4.7 ± 0.6			3.695 ± 0.659	60.1 ± 9.8

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A 75	B -75
	2	A 156	B -158
	3	A 263	B -258

Paraph		Filter		
		0.1		0
		0.3		15
Date	14-Mar-05	0.6		50
		0.8		89
		1		141

RDF/BCO/18

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	α-octanol [111-87-5]		
Batch No.	S02961-454		
Concentration	100%	Treatment time	10 min
Code	B4	(14)	
Sequence	2005/ intern2 kalveren	OP-KIT	

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.084	1.3
2	NaCl 0.9%	0	2	2	0.085	3.3
3	100%	1	1	0	0.036	0.5
Mean ± S.D.		0.7 ± 1.2			0.068 ± 0.028	1.7 ± 1.4
					Corrected value	Corrected value
13	Test article	1	7	6	5.3	23.2
14		0	18	18	17.3	44.8
15	100%	0	9	9	8.3	31.8
Mean ± S.D.		10.3 ± 6.2			1.533 ± 0.322	33.3 ± 10.9

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A 75	B -75
	2	A 155	B -161
	3	A 259	B -261

Paraph		Filter			
		0.1		1	
		0.3		16	
Date	01-Mar-05	0.6		51	
		0.8		91	
		1		143	

RDF/BCO/18

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	2-ethyl-1-hexanol [107-76-7]		
Batch No.	S01263-011	Treatment time	10 min
Concentration	100%	Code	A4 (15)
Sequence	2005/ intern I kalveren	OP-KIT	

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.023	0.3
2	NaCl 0.9%	0	0	0	0.069	1.0
3	100%	0	0	0	0.044	0.7
Mean ± S.D.		0.0 ± 0.0			0.045 ± 0.023	0.7 ± 0.4
					Corrected value	Corrected value
13	Test article	0	4	4	4.0	11.2
14		0	0	0	0.0	5.3
15	100%	0	3	3	3.0	29.4
Mean ± S.D.		2.3 ± 2.1			0.864 ± 0.777	15.3 ± 12.6

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A	75 B -75
	2	A	157 B -161
	3	A	260 B -259

Paraph		Filter			
		0.1	1		
		0.3	16		
Date	28-Feb-05	0.6	50		
		0.8	88		
		1	140		

RDF/BCO/18

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	1-Hexanol [111-27-3]		
Batch No.	A020123401		
Concentration	98%	Treatment time	10 min
Code	D2	16	
Sequence	2005/ Intern3 kalveren	OP-KIT	

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.008	0.1
2	NaCl 0.9%	0	0	0	0.026	0.4
3	100%	0	0	0	0.006	0.1
Mean ± S.D.		0.0 ± 0.0			0.013 ± 0.011	0.2 ± 0.2
					Corrected value	Corrected value
10	Test article	0	16	16	16.0	70.2
11	100%	0	13	13	13.0	61.3
12		0	13	13	13.0	73.2
Mean ± S.D.		14.0 ± 1.7			3.615 ± 0.398	68.2 ± 6.2

NC: Negative Control

REMARKS	Filter	A	B	OPACITY
	1	A	B	75 -75
	2	A	B	158 -160
	3	A	B	256 -258

Paraph

Date 07-Mar-05

Filter		
0.1	1	
0.3	15	
0.6	50	
0.8	90	
1	145	

RDF/BCO/18

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	Acetone [67-64-1]		
Batch No.	442942/1		
Concentration	100%	Treatment time	10 min
Code	A5	17	
Sequence	2005/ intern 1 kalveren	OP-KIT	

No. Cornea	Treatment	Opacity at			Permeability	In vitro score			
		t0	t120	t120 - t0					
1	NC	0	0	0	0.023	0.3			
2		0	0	0	0.069	1.0			
3	100%	0	0	0	0.044	0.7			
Mean ± S.D.		0.0 ± 0.0			0.045 ± 0.023	0.7 ± 0.4			
		Corrected value				Corrected value			
16	Test article	0	101	101	101.0	2.824	2.779	142.7	
17	100%	0	92	92	92.0	2.452	2.407	128.1	
18		0	81	81	81.0	3.428	3.383	131.7	
Mean ± S.D.		91.3 ± 10.0				2.856 ± 0.493		134.2 ± 7.6	

NC: Negative Control

REMARKS	Filter	OPACITY	
		A	B
	1	75	-75
	2	157	-161
	3	260	-259

Paraph		Filter		
		0.1	1	
		0.3	16	
Date	28-Feb-05	0.6	50	
		0.8	88	
		1	140	

RDF/BCO/18

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	cyclohexanol		
Batch No.	S05238-044		
Concentration	100%	Treatment time	10 min
Code	B5	(18)	
Sequence	2005/ intern2 kalveren	OP-KIT	

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.084	1.3
2	NaCl 0.9%	0	2	2	0.085	3.3
3	100%	1	1	0	0.036	0.5
Mean ± S.D.		0.7 ± 1.2			0.068 ± 0.028	1.7 ± 1.4
					Corrected value	Corrected value
16	Test article	1	13	12	11.3	36.8
17		0	12	12	11.3	38.7
18	100%	0	13	13	12.3	55.4
Mean ± S.D.		11.6 ± 0.6			2.132 ± 0.644	43.6 ± 10.2

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A 75	B -75
	2	A 155	B -161
	3	A 259	B -261

Paraph	Filter
	0.1 1
	0.3 16
Date	01-Mar-05
	0.6 51
	0.8 91
	1 143

RDF/BCO/18

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	Cetylpyridinium bromide (6%) [140-72-7]		
Batch No.	038H2509		
Concentration	6%	Treatment time	10 min
Code	C5		
Sequence	2005/Intern4 kalverogen		OP-KIT

No.	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.045	0.7
2	NaCl 0.9%	0	0	0	0.022	0.3
3	100%	0	0	0	0.012	0.2
Mean ± S.D.		0.0 ± 0.0			0.026 ± 0.017	0.4 ± 0.3
Corrected value					Corrected value	
13	Test article	0	20	20	2.252	53.4
14		0	13	13	1.879	40.8
15	100%	0	12	12	0.919	25.4
Mean ± S.D.		15.0 ± 4.4			1.657 ± 0.688	39.9 ± 14.0

NC: Negative Control

REMARKS	Filter	OPACITY	
		A	B
	1	75	-75
	2	156	-158
	3	263	-258

Paraph	Filter	
	0.1	0
	0.3	15
Date	0.6	50
	0.8	89
	1	141

RDF/BCO/18

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	Benzalkoniumchloride [8001-54-5]		
Batch No.	033K2544		
Concentration	10g/g%	Treatment time	10 min
Code	C3 20		
Sequence	2005/Intern4 kalverogen	OP-KIT	

No. Cornea	Treatment	Opacity at			Permeability	In vitro score
		t0	t120	t120 - t0		
1	NC	0	0	0	0.045	0.7
2	NaCl 0.9%	0	0	0	0.022	0.3
3	100%	0	0	0	0.012	0.2
Mean ± S.D.		0.0 ± 0.0			0.026 ± 0.017	0.4 ± 0.3
					Corrected value	
7	Test article	0	115	115	115.0	174.9
8	100%	0	95	95	95.0	152.5
9		0	107	107	107.0	172.0
Mean ± S.D.		105.7 ± 10.1			4.050 ± 0.255	166.5 ± 12.2

NC: Negative Control

REMARKS	Filter	OPACITY	
		A	B
	1	75	-75
	2	156	-158
	3	263	-258

Paraph		Filter	0.1	0
			0.3	15
Date	14-Mar-05		0.6	50
			0.8	89
			1	141

RDF/BCO/18